WorkBeads™ Protein A

Media for laboratory and large scale purification of proteins.

Product name	Pack size	Article number
WorkBeads Protein A	Bulk Media – 1.5 ml	40605001
WorkBeads Protein A	Bulk Media – 5 ml	40605002
WorkBeads Protein A	Bulk Media – 10 ml	40605003
WorkBeads Protein A	Bulk Media – 100 ml	40605004
WorkBeads Protein A	Bulk Media – > 1L inquiry	40605005

Formats of use

Beads are designed for purification of mono- and polyclonal antibodies. For lab scale work and initial screening in process development we recommend our pre-packed columns BabyBio A 1 ml and 5 ml containing WorkBeads Protein A medium. For research other formats can be used such as test tube batch adsorption, spin columns, gravity columns or multiwell filter plates. The medium can be used for immunoprecipitation experiments.

Column packing

A simplified protocol for column packing of WorkBeads Protein A is given below. Also follow the instructions of the column manufacturer. For best packing result use a packing reservoir or an adaptor for column-to-column connection. This should give a total volume big enough to allow filling the column with the gel slurry in one go, which improve the packing quality. For lab columns the following points can be used as brief instruction:

1. Transfer enough medium (gel) to a No 3 glass filter on a vacuum flask, and wash it using 1-2 bed volumes of water three times by vacuum suction. Let suction dry briefly.
2. Prepare a ca 65% gel slurry Weigh in 1.25 g medium per ml packed bed to a suitable container. Add 0.85 ml water per ml packed bed and resuspend the gel. For a 10-ml column bed, weigh in 12.5 g gel and add 7.5 ml water.
3. Close the bottom end of the column and apply the packing device on top. Pump water through the upper adaptor to remove air.
4. Pour the suspension into the column in one go (with the packing device attached), and add extra water if needed to fill the column to the top. Slightly, open the connection between the pump and adaptor to let water out during insertion of the adaptor. Apply the top adaptor and seal the connection. Open the bottom end of the column.

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5. Start a flow of 300 cm/h to allow the medium to form a bed. Run the flow until the bed height is constant. Before stopping the flow make a mark at the top of the bed. Stop the flow. (The bed may spring back slightly when the flow is stopped.)
6. Close the bottom adaptor, and remove the top adaptor and the packing tube.
7. If the bed height (at the mark) differs from the desired, carefully resuspend and remove gel from the top of the bed to adjust, or if needed replace water with gel slurry to obtain the desired bed height. Allow for ca 4-5 mm gel above the mark.
8. Fill the top of the column with water and apply the top adaptor without leaving air in the column. The connection of the adaptor to the system or pump must be released. Push down the adaptor to the mark. Reconnect the top adaptor to the system and open the bottom end of the column. The column is ready for use.
9. Store the packed column at +2°C to +80°C, filled with 20% ethanol.

Instructions

The following brief instruction gives general conditions for purification using a column packed with WorkBeads Protein A. See the tips below for optimization.

Sample preparation

Clarify the sample by centrifugation at 10,000-20,000 × g for 15-30 minutes. It is recommended to also pass the sample through a 0.22-0.45-µm filter to remove any remaining particles. If the sample contains only small amounts of particles it may be enough to only perform filtration. Make sure that the sample has a pH between 5 and 8. Preferably the sample should have the same pH and ionic strength as the Binding buffer.

Standard purification

1. Equilibrate the column using 10 column volumes (CV) of PBS, pH 7.4 (Binding buffer).
2. Apply a clarified sample under neutral conditions.
3. Wash using 10-20 CV Binding buffer.
4. Elute with 5 CV 100 mM Na-citrate, pH 3 (Elution buffer). Include 100 µl 1 M tris-HCl, pH 9 per 1 ml collected fraction.
5. Reequilibrate with 10 CV Binding buffer.
6. Equilibrate with 10 CV 20% ethanol for storage. Close the column using the included lids.

Before a purification run it is recommended to make a blank run (no sample application) to remove any contaminants that may exist on the column. Do this also for a freshly packed column. Although the above standard conditions usually give excellent results it may be worthwhile to make optimization of the purification protocol for optimal purification results. See below for more details.

Optimization

Selection of column size

The column size should be selected based on estimated amount of target protein in each run, and the dynamic binding capacity (DBC) of the column. DBC is the capacity under the chosen run conditions and is usually higher than the static binding capacity (total binding capacity). Figure 1 shows example of DBC at different flow rate (or residence time). At a low flow rate the capacity is high. At increased flow rate the binding capacity decreases. To obtain the highest possible recovery of target protein

we recommend application of no more sample than to use 80% of the capacity of the column at the selected flow rate. Consider using a larger column or dividing the sample into repeated purification cycles. If the amount of IgG in the sample is not known it can be determined by running a small sample on a BabyBio 1 ml and determine the amount of IgG that can be eluted. Alternatively, make the purification and collect the flow-through material for subsequent analysis to determine whether the column was over-saturated. If desired the collected flow-through material can be reapplied on the column after proper regeneration, in a separate purification. Any remaining IgG should be captured.

Binding capacity

The IgG binding capacity depends on the flow rate used for binding. The binding capacity is more than 40 mg human serum IgG/ml medium at 2.5 minutes residence time in a 6.6 x 100 mm column, which corresponds to a linear flow rate of 240 cm/min. Such dynamic binding capacity may differ between IgG species. The binding capacity decreases upon increase in flow rate, see figure 1. We therefore recommend flow rates of 240 cm/min or lower during sample application. If desired runs can be done much faster in small column for analytical amounts of IgG, since the binding capacity is more than needed.

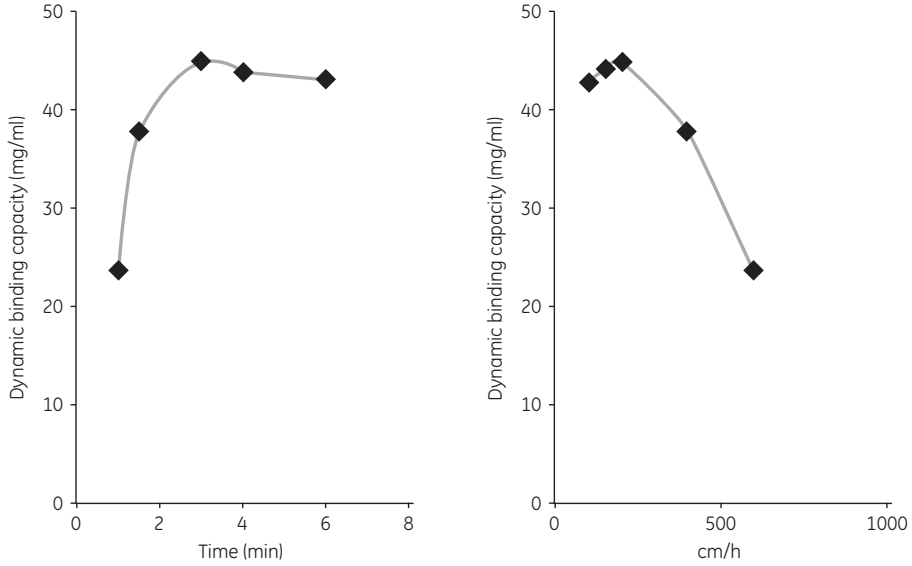

Figure 1. DBC at 10% ($Q_{B,10}$) for human serum IgG at different flow rates determined by frontal analysis of 1 mg/ml IgG in PBS, pH 7.4 in a 6.6 x 100 mm bed.

Optimization of binding

Human IgG and IgG from several other species binds to WorkBeads Protein A under neutral pH at moderate salt concentrations, for example:

- 20 mM Na-phosphate, 150 mM NaCl, 7.4 (PBS)
- 50 mM Na-phosphate, pH 7.4.
- 50 mM Na-borate, pH 9

For weakly bound IgG a combination of high pH and ionic strength may be needed for binding, for example:

- 50 mM Na-borate, 4 M NaCl, pH 9.

Extra wash step

To remove weakly adsorbed impurities it may be useful to add an extra washing step after the standard wash with the Binding buffer. This can be done using a buffer with slightly increased ionic strength compared to the Binding buffer or by a small decrease in pH that do not elute the target protein, see pH optimization below.

Optimization of elution

IgGs can be sensitive to low pH. There are several ways to avoid denaturation:

- Add 100 µl of 1 M Tris-HCl, pH 9 neutralization buffer per ml to each fractionation tube before the purification.
- Immediately after fractionation collect the target protein and perform buffer exchange using a BabyBio Dsalt column (see the product list) equilibrated with a neutral buffer.
- Perform gradient elution using a gradient from 100 mM Na-citrate, pH 6.0 to 100 mM Na-citrate, pH 3.0 over 10-20 CV. Desorption will occur when the pH is low enough, while avoiding very low pH.
- Make a test gradient-elution run (as above) with a small amount of sample to determine at what pH the target protein is eluting. The pH measured at the tail of the peak can be selected for elution. Prepare a 100 mM Na-citrate-buffer with the selected elution pH and make the scale-up using this Elution buffer.

Additional purification step (Polishing step)

Antibody purification on WorkBeads Protein A gives high purity in a single step. For very high requirement on purity it may be necessary to add a second purification step. This step is used to remove traces of leaked of protein A ligand and remaining impurities from the sample. In fact, an added polishing step may allow omission of optimization of the WorkBeads Protein A protocol also at very high purity requirements. The polishing purification step can be based on several chromatographic techniques:

Size-exclusion chromatography (gel filtration)

Dimers or aggregates of the antibody and complexes of leaked-off protein A and antibody will be eluted earlier than monomeric antibodies on a SEC column under neutral conditions. This technique is simple to set up and is recommended for high purity demands in lab scale purification. Optimization is often not required for significant purification, but may sometimes be worthwhile. The technique is not recommended for bioprocess scale applications due to dilution effects and low capacity.

Cation exchange chromatography

Most antibodies are weakly basic and will bind to the column. Protein A does not bind under neutral conditions. Removal of protein A requires that the conditions used allow dissociation of the protein A and the antibody. Optimization is usually required.

Anion exchange chromatography

Protein A and complexes between protein A and antibody tend to bind to anion exchange columns at neutral pH, whereas antibodies usually do not bind under the same conditions. The technique is sometime referred to as negative chromatography.

Maintenance of the column

Storage

The WorkBeads Protein A media are supplied as aqueous suspensions containing 20% ethanol as preservative. The media should be stored at +2 to +80C. Between uses wash the column using 20% ethanol, and close it using lids at the inlet and outlet.

Cleaning using NaOH

Most samples contain small amounts of impurities that tend to adsorb to the column by unspecific interactions. Collecting such material may reduce the performance of the column over time. It is therefore common to make regular cleaning of the column. Cleaning-in-place (CIP) using NaOH is the most common method, although prolonged wash with alkaline conditions will reduce the functionality of the column and must therefore be kept to a minimum (see Figure 2). After purification perform the following steps:

1. Unless elution was done at very low pH there may be a need for regeneration by cleaning the column with, for example, 10 CV100 mM Gly-HCl, pH 2.7 or 100 mM Na-citrate, pH 3).
2. Wash the column with 5 CV water.
3. CIP by passing 15 CV 15 mM NaOH over 15 minutes, or for more harsh conditions 50 mM NaOH. For increased efficiency, before the NaOH wash, include a passage of 15 CV 100 mM 1-thioglycerol, pH 8.5, over 15 minutes to reduce any oxidized aggregates adsorbed to the column.
4. Wash with 10 CV neutral buffer. Make sure that neutral pH is restored in the column. Prolonged exposure to extreme pH may harm the medium.
5. Wash with 10 CV water.
6. Wash with 10 CV 20% ethanol before storage.

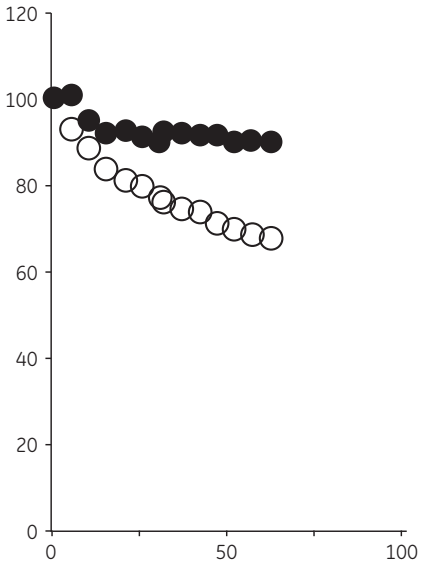

Figure 2. Effect of incubation with NaOH. Multiple cycles of cleaning with 15 CV 100 mM 1-thioglycerol and 15 CV NaOH (each cycle) was performed. At start and after every fifth cycle DBC was determined as described earlier. Closed circles, 15 mM NaOH and open circles, 100 mM NaOH. Values are given in percent remaining DBC at 2.5 minutes residence determined a described in Figure 1.

Cleaning using strong denaturants

Guanidine hydrochloride and urea can be used for CIP in lab scale work. For industrial scale CIP these compounds are avoided because of cost and negative environmental effects when used in large quantities.

1. Unless elution was done at very low pH there may be a need for regeneration by cleaning the column with, for example, 10 CV100 mM Gly-HCl, pH 2.7 or 100 mM Na-citrate, pH 3).
2. Wash the column with 5 CV water.
3. CIP by passing 10 CV 6 M urea or 6 M guanidine hydrochloride over more than 1 hour. Alternatively, stop the flow and incubate overnight.
4. Wash with 10 CV water.
5. Wash with 10 CV 20% ethanol before storage.

Intended use

WorkBeads Protein A is intended for research and for process development. WorkBeads Protein A shall not be used for preparation of material for clinical or diagnostic purposes. ????

Safety

Please read the MSDS for WorkBeads Protein A, and the safety instructions for any equipment to be used for the purification.

Characteristics

Medium	WorkBeads Protein A
Target substance	Antibodies (IgG), bound via the Fc-region;
Matrix	Rigid, highly cross-linked agarose
Ligand	Recombinant protein A expressed in *E. coli* using animal-free medium
Coupling chemistry	Bromohydrin
Dynamic binding capacity (DBC)[1]	>40 mg human IgG/ml medium
Recommended flow rate[2]	250 cm/min
Max flow rate[3]	500 cm/min
Chemical stability	Compatible with all standard aqueous buffers used for protein purification, and 10 mM HCl (pH 2),10 mM NaOH (pH 12), 0.1 M sodium citrate-HCl (pH 3), 6 M guanidine-HCl, 20% ethanol. Should not be stored at low pH for prolonged time.
Recommended working range pH Stability	3-10 short term 2-12 cleaning
Storage	+2°C to +8°C in 20% ethanol

[1] DBC was determined at 10% breakthrough ($Q_{B,10}$) by frontal analysis with 1 mg/ml human serum IgG in PBS, pH 7.4 at 240 cm/h in a column with a WorkBeads Protein A bed of 6 x 100 mm (=2.5 minutes residence time).

[2] At 20 °C using aqueous buffers.

[3] At 20 °C using aqueous buffers in 10 x 300 mm column bed. Decrease the max flow if the liquid has a higher viscosity. Higher viscosities can be caused by low temperature (use max flow/2 at 4 °C), or by additives (e.g, use max flow/2 for 20% ethanol). For large columns a lower max flow should be applied.

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